PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Valerie CHEYNET-SAUVION et al.

Application No.: 09/402,131

Filed: December 8, 1999 Docket No.: 104458

For: RNA-DEPENDENT RNA POLYMERASE FUNCTIONING PREFERABLY ON RNA MATRIX AND PROMOTER-DEPENDENT TRANSCRIPTION PROCESS WITH SAID RNA-DEPENDENT RNA POLYMERASE (AS AMENDED)

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE TITLE:

Please change the title to --RNA-DEPENDENT RNA POLYMERASE FUNCTIONING PREFERABLY ON RNA MATRIX AND PROMOTER-DEPENDENT TRANSCRIPTION PROCESS WITH SAID RNA-DEPENDENT RNA POLYMERASE--.

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 3, line 6, delete "[sic]".

Page 15, line 13, change "(1922)" to --(1992)--.

IN THE CLAIMS:

Please amend claims 1-32 as follows:

1. (Amended) [Method] A method of amplifying [any] an RNA target sequence, by transcription under the control of a promoter, in an RNA sample comprising said target sequence,

[in which] said method comprising bringing said sample [is brought] into contact:

- with a reagent capable of hybridizing with [said] RNA comprising said target sequence,

- in the absence of deoxyribonucleoside triphosphates,

- and with an enzymatic system comprising an RNA-dependent RNA polymerase activity, under conditions allowing the hybridization of said reagent with said RNA comprising said target sequence and under conditions allowing the functioning of said RNA-dependent RNA polymerase activity;

[in which] wherein said reagent contains:

(i) a first nucleotide strand comprising: a) a first nucleotide segment capable of playing the role of sense strand of a promoter for said RNA polymerase activity and b), downstream of said first segment, a second nucleotide segment comprising a sequence capable of hybridizing with a region of said RNA, and

(ii), in the hybridized state on the first strand, a second nucleotide strand comprising a third nucleotide segment capable of hybridizing with said first segment so as to form with it a functional double-stranded promoter;

and [in which] wherein said RNA polymerase activity is capable of transcribing an RNA template, in the presence of said reagent hybridized with said template, in the absence of associated protein factor and in the absence of a ligase activity.

2. (Amended) [Method] A method according to claim 1, [in which] wherein said third segment is flanked, at its upstream end, by a fourth nucleotide segment which is shorter than said second segment of the first strand.

3. (Amended) [Method] A method according to claim 2, [in which] wherein said fourth segment is capable of hybridizing with a portion opposite said second segment.

4. (Amended) [Method] A method according to [either of claims 2 and 3, in which] claim 2, wherein said fourth segment of said second strand is chosen from those whose sequence facilitates the initiation of transcription for said RNA polymerase.

5. (Amended) [Method] A method according to [any one of claims 2 to 4, in which] claim 2, wherein said second segment of said first strand contains a number of nucleotides at least equal to the sum of the number of nucleotides of said fourth segment, if it is present, and of the number of nucleotides of said sequence of the second segment which is capable of hybridizing with said region of said RNA.

6. (Amended) [Method] A method according to [any one of the preceding claims, in which] claim 1, wherein said first and third segments consist of DNA.

7. (Amended) [Method] A method according to [any one of the preceding claims, in which] claim 1, wherein said fourth segment consists of DNA.

8. (Amended) [Method] A method according to [any one of the preceding claims, in which] claim 1, wherein said RNA polymerase is a virus or phage wild-type RNA polymerase.

9. (Amended) [Method] A method according to claim 8, [in which] wherein said polymerase is chosen from the family of RNA polymerases including [the] T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

10. (Amended) [Method] A method according to claim 8, [in which] wherein said RNA polymerase is derived by mutation from an RNA polymerase chosen from the family of RNA polymerases including [the] T7, T3 and SP6 RNA polymerases.

11. (Amended) [Method] A method according to claim 10, [in which] wherein said RNA polymerase contains at least one mutation in the region corresponding to the T7 RNA polymerase sequence containing amino acids 625 to 652.

12. (Amended) [Method] A method according to claim 11, [in which] wherein said RNA polymerase is capable of transcribing a polynucleotide target sequence with a better yield when said target sequence consists of RNA than when it consists of DNA.

13. (Amended) [Method] A method according to [any one of the preceding claims, in which] claim 1, wherein said enzyme system contains only [an] RNA polymerase activity.

14. (Amended) An RNA polymerase [which can be used in the method of any one of the preceding claims], capable of transcribing, under the control of a promoter, a polynucleotide target of interest of [any] a sequence contained in a polynucleotide template, by synthesizing, in the presence of said template and in the absence of associated protein factor, a product of transcription containing an RNA sequence complementary to said sequence, and said RNA polymerase being capable of synthesizing said product of transcription with a better yield when said target sequence of said template consists of RNA than when it consists of DNA.

15. (Amended) An RNA polymerase according to [the preceding] claim 14, [in which] wherein the ratio of the yield of product of transcription of the RNA template to the yield of product of transcription of the DNA template is greater than 2 [and in particular greater than 10].

16. (Amended) An RNA polymerase according to [either of claims 14 and 15] claim 14, [characterized in that it] wherein said RNA polymerase is derived by mutation from a virus or phage RNA polymerase.

17. (Amended) An RNA polymerase according to claim 16, [characterized in that] wherein said phage is an E.coli phage.

18. (Amended) An RNA polymerase according to [any one of claims 14 to 17] claim 14, [characterized in that it] wherein said RNA polymerase possesses a protein sequence homology greater than 50%[, and in particular greater than 80%] with a wild-type RNA polymerase of the family of DNA-dependent RNA polymerases including [the] T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.

19. (Amended) An RNA polymerase according to claim 18, [characterized in that it] wherein said RNA polymerase contains at least one mutation in a region corresponding to the T7 RNA polymerase sequence containing amino acids 625-652.

20. (Amended) An RNA polymerase according to claim 19, [characterized in that it] wherein said RNA polymerase has the composition of a wild-type DNA-dependent RNA polymerase, [with the exception of the fact] except that it contains at least one mutation in said region.

21. (Amended) An RNA polymerase according to claim 19, [or 20, characterized in that it] wherein said RNA polymerase contains at least one mutation at a position corresponding to one of positions 627, 628, 631, 632 and 639 of the T7 RNA polymerase amino acid sequence.

22. (Amended) An RNA polymerase according to [any one of claims 19 to 21] claim 19, [characterized in that] wherein said mutation comprises the replacement of an

amino acid residue, [chosen] selected from the group consisting of arginine, lysine, serine and tyrosine, of the wild-type RNA polymerase, with another amino acid residue.

23. (Amended) An RNA polymerase according to claim 22, [characterized in that] wherein said amino acid replaced is an arginine or a lysine and/or [in that] wherein said other amino acid residue is an alanine, valine, leucine, isoleucine, glycine, threonine or serine residue.

24. (Amended) An RNA polymerase according to [any one of claims 19 to 23] claim 19, [characterized in that] wherein said mutation comprises the replacement of all or part of said region with a homologous region present in a wild-type RNA-dependent polymerase.

25. (Amended) [Gene] A gene encoding an RNA polymerase as defined in [any one of claims 14 to 24] claim 14.

26. (Amended) [Expression] An expression vector into which a gene as defined in [the preceding] claim 25 is inserted, said vector being capable of expressing said RNA polymerase in a host cell.

27. (Amended) [Host] A host cell containing an expression vector as defined in [the preceding] claim 26.

28. (Amended) [Method] A method of producing an RNA polymerase as defined in [any one of claims 14 to 24, characterized in that] claim 14, said method comprising: a) obtaining a gene encoding a wild-type RNA polymerase [is obtained in a known manner], b) performing at least one mutation [is performed] on said gene, c) inserting the mutated gene obtained [is inserted] into an expression vector, d) expressing said vector [is expressed] in a host cell in order to obtain a mutated RNA polymerase, and e) among the mutated RNA

polymerases obtained, selecting those which exhibit at least one of the properties of [an] said RNA polymerase [as defined in either of claims 14 and 15 are selected] to be produced.

29. (Amended) [Use of] A method of transcription of a template strand comprising an RNA target sequence, said method comprising bringing the template strand into contact with an RNA polymerase capable of transcribing an RNA template, under the control of a promoter, in the absence of auxiliary protein factor, [in a method of transcription of a template strand comprising an RNA target sequence, in which] wherein said RNA polymerase is [chosen] selected from the group consisting of T7 RNA polymerase, [the] SP6 RNA polymerase and the RNA polymerases as defined in [any one of claims 14 to 24] claim 14.

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30. (Amended) [Use of] A method of transcription of a template strand comprising an RNA target sequence, said method comprising bringing the template strand into contact with an RNA polymerase capable of transcribing an RNA template, under the control of a promoter, in the absence of auxiliary protein factor, [in a method of transcription of a template strand comprising an RNA target sequence, in which] wherein said template strand consists of: (a) RNA from one of positions +1 to +5 up to the 5' end of the template strand, and [consists of] (b) DNA from said position up to the 3' end of the template strand when said 3' end does not coincide with said position.

31. (Amended) [Use] A method according to [the preceding] claim 30, [in which] wherein said RNA polymerase is a virus or phage wild-type RNA polymerase.

32. (Amended) [Use] A method according to [the preceding] claim 31, [in which] wherein said RNA polymerase is chosen from T7, T3 and SP6 RNA polymerase.

Please add new claims 33 and 34 as follows:

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--33. An RNA polymerase according to claim 15, wherein said ratio is greater than 10.--

--34. An RNA polymerase according to claim 18, wherein said RNA polymerase possesses a protein sequence homology greater than 80% with the wild-type RNA polymerase of said family of DNA-dependent RNA polymerases including T7 RNA polymerase, T3 RNA polymerase and SP6 RNA polymerase.--

REMARKS

Claims 1-34 are pending. This Preliminary Amendment amends the specification and claims 1-34 and adds new claims 33 and 34. These amendments correct typographical errors in the specification and remove multiple dependencies in the claims.

Prompt and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

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